

of SSA 939211
Jms.

Salk Inst.
San Diego, Calif.

NSG-7627

17P.

5317-IN

Stereoselective Formation of a 2'(3')-Aminoacyl Ester of a Nucleotide

Arthur L. Weber

The Salk Institute for Biological Studies
San Diego, California 92138, USA

(NASA-CR-176830) STEREOSELECTIVE FORMATION N86-26373
OF A 2 PRIME (3 PRIME)- AMINOACYL ESTER OF A
NUCLEOTIDE (Salk Institute for Biological
Studies) 17 p HC A02/MF A01 CSCL 07A
G3/23 43081

Send reprints to:

A.L. Weber
The Salk Institute For Biological Studies
P.O. Box 85800
San Diego, CA. 92138 USA

Summary. Reaction of DL-serine and adenosine-5-phosphorimidazolidine in the presence of adenosine-5'-(O-methylphosphate) and imidazole resulted in the stereoselective synthesis of the aminoacyl nucleotide ester, 2'(3')-O-seryl-adenosine-5'-(O-methylphosphate). The enantiomeric excess of D-serine incorporated into 2'(3')-O-seryl-adenosine-5'-(O-methylphosphate) was about 9%. Adenylyl-(5'->N)-serine and an unknown product also incorporated an excess of D-serine; however, seryl-serine showed an excess of L-serine. The relationship of these results to the origin of the biological pairing of L-amino acids and nucleotides containing D-ribose is discussed.

Key words: Stereoselection - Amino acid activation - Aminoacyl nucleotide ester - Prebiotic chemistry - Origin of optical activity - Nucleotide - Molecular Evolution.

Abbreviations: ImpA, adenosine-5'-phosphorimidazolidine; MepA, adenosine-5'-(O-methylphosphate); ser, serine; MepA-ser, 2'(3')-O-(seryl)-adenosine-5'-(O-methylphosphate); ser-ser, serylserine; ser-N-pA, adenylyl-(5'->N)-serine; Ado, adenosine; Im, imidazole; ser-Im, N-(seryl)imidazole; pA, adenosine-5'-monophosphate; ser-pA, seryl adenylate anhydride.

Introduction

Contemporary organisms use L-amino acids in proteins and nucleotides containing D-ribose (D-nucleotides) in nucleic acids. The origin of this pairing of L-amino acids and D-nucleotides can be viewed as having occurred in two steps. In the first step, generally called the origin of optical activity, either the L-amino acid or D-nucleotide was selected by amplification of what was initially a small excess of enantiomer (Profy and Usher 1984a and references therein). In the next step, either the chiral L-amino acids caused the selection of D-nucleotides, or conversely, D-nucleotides brought about the selection of L-amino acids. This second step, which could be called the chiral pairing of amino acids and nucleotides, may have been caused by the chemical difference between the L-amino acid, D-nucleotide pair and its diastereomeric L-amino acid, L-nucleotide pair or D-amino acid, D-nucleotide pair.

Although there have been some proposals of stereoselective interaction between polypeptides of L-amino acids and polynucleotides of D-ribose (Lacey and Pruitt 1969; Carter and Kraut 1974), only recently has chiral pairing been approached experimentally by examining the stereoselective acylation of IpI and polyribonucleotides by N-protected DL-aminoacyl imidazolides and DL-aminoacyl imidazolides (Profy and Usher 1984 a,b; Usher and Needels 1984; Usher et al. 1984). Although the acylation of the 2'-(3')-hydroxyl groups of IpI did not show significant stereoselection, the acylation of the internal 2'-hydroxyl of IpI by N-(*tert*-butoxycarbonyl)-DL-alanine imidazolidine gave an enantiomer excess of the L-isomer, and the acylation by DL-alanine imidazolidine yielded an enantiomeric excess of the D-isomer. Likewise, acylation of the 2'-hydroxyl groups of

polyribonucleotides by N-(3,5-dinitrobenzoyl)-DL-alanine(or leucine) imidazolide showed a preference for the L-isomer, and the aminoacylation by leucine imidazolide showed a preference for the D-isomer.

We now report the stereoselective aminoacylation of the 2'(3')-hydroxyl groups of a nucleotide. The aminoacylation was carried out by reacting DL-ser with the phosphoanhydride, ImpA, in the presence of imidazole and MepA as an aminoacyl acceptor. We had previously studied this type of reaction as a chemical model of the aminoacylation of tRNA (Weber and Orgel 1978a).

Experimental

Materials. L-Serine($[\alpha]_D^{20}$ -7.4, $c = 2$, H_2O ; lit. $[\alpha]_D^{25}$ -7.5 from Greenstein and Wintz (1961), DL-serine($[\alpha]_D^{20}$ <0.024), and adenosine 5'-monophosphate from Sigma Chemical Co.; imidazole (recrystallized) from J.T. Baker Chemical Co.; seryl-serine from Vega-Fox Biochemicals; triphenylphosphine and Aldrithiol from Aldrich Chemical Co. and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide from Story Chemical Corp.; L-[G- 3H]serine and D-[3- ^{14}C]serine from ICN Chemical and Radioisotope Division.

MepA was prepared from adenosine-5'-phosphate and methanol as described by Lohrmann and Orgel (1978). ImpA was synthesized by a modification of the procedure of Mukaiyama and Hashimoto (1971).

Chromatography and Electrophoresis. Paper chromatography was carried out by descending elution on Whatman 3MM paper in System 1 with isopropanol-triethylamine-water (7:1:2,v/v/v), and in System 2 with n-butanol-5M acetic acid (2:1, v/v). High-voltage paper electrophoresis on Whatman 3MM paper used a buffer of

0.05 M formic acid adjusted to pH 2.7 with concentrated ammonium hydroxide in System 3, and a buffer of 0.03 M potassium phosphate (pH 7.1) in System 4. The products formed from [^3H] and [^{14}C]serine were located by running the chromatograms and electrophoretograms through a Baird RSC-363 radiochromatographic scanner. Table 1 lists the chromatographic and electrophoretic mobilities of the substances studied. Reaction products were identified by co-chromatography and co-electrophoresis with commercially available standards whenever possible. The ninhydrin spray was used for detecting amino acids and their derivatives. Nucleoside-containing products were visualized under UV light.

← Insert
Table 1

Characterization of Reaction Products. The identity of MepA-ser is supported by its hydrolytic behavior which resembles that of other 2'-(3')-amino acid esters of nucleotides (Zachau and Feldmann 1965; Schuber and Pinck 1974; Weber and Orgel 1978b). MepA-ser hydrolyzed to MepA and ser under weakly alkaline conditions (pH 10, 1 hour, ambient temperature). It was stable in weak acid (pH 3) for the same length of time. The chromatographic (System 2) and electrophoretic (System 3) mobility of MepA-ser was similar to 2'-(3')-glycyl ester of MepA (Weber and Orgel 1978b).

The structure of ser-N-pA is supported by its hydrolytic behavior which resembles that of other amino acid phosphoramidates (Clark et al. 1966). Ser-N-pA hydrolyzed to pA and ser under weakly acidic conditions (pH 2.5, 18 hours, ambient temperature). It was stable under weakly basic conditions (pH 10) for the same length of time. The chromatographic (System 1) and electrophoretic mobility (System 3) of ser-N-pA was similar to the glycine phosphoramidate of pA (Weber 1978a; Lohrmann et al. 1975).

Reaction of Serine with ImpA in the Presence of MepA and Imidazole. MepA (15 mg, 37 μ mole) was dissolved in a mixture of 75 μ l 3.0 M imidazole (pH 5.8), 74.5 μ l of 0.50 M DL-serine, and 13.1 μ l of 0.02 M L-serine. Next, 18.8 μ l of 2.0 M NaOH was added in order to bring the pH back to 5.8. The solution was then dried overnight in a desiccator over P_2O_5 in vacuo. Twenty-four microliters of D-[^{14}C]serine (15 μ Ci, 55 mCi/mmole), 7.2 μ l of L-[3H]serine (45 μ Ci, 5 Ci/mmole), and 40 μ l of water were added to the residue to give a final volume of 100 μ l. The reaction of serine with ImpA was started by adding 66.7 μ l of this serine solution to solid ImpA (2.9 mg, 6.7 μ moles). The remaining 33.3 μ l of the serine solution provided a control reaction without ImpA. The temperature of the reaction and the control was maintained at 20°C. At time intervals 5 μ l aliquots were removed from the reaction and the control solutions. These aliquots were added to 40 μ l of 0.25 M formic acid and frozen at -80°C until analyzed.

The reaction products were separated by electrophoresis of a 15 μ l aliquot of these solutions in System 1. The products formed from radioactive serine were located by scanning the electrophoretograms with a Baird Atomic RSC-363 radiochromatographic scanner. The areas of the electrophoretograms that contained radioactive products were cut out and placed in elution tubes. They were wetted with 1.0 ml of 1.0 M ammonium hydroxide. After 20 min., the elution tubes were centrifuged in order to elute radioactive substances on the paper. This elution process was repeated twice more with about 1 ml of water. Each eluent was then dried in vacuo on a Buchler Evapo-mix and the residue redissolved in 1.0 ml H_2O . An 0.80 ml aliquot of each solution was added to 20 ml of Aquasol-2 (New England Nuclear) in a scintillation vial and counted in a Beckman scintillation counter. Each vial was counted six times for 20 minutes. The percent yields of D-[^{14}C] serine and L-[3H] serine in each product were

calculated from the cpm appearing in the ^3H and ^{14}C channels of the scintillation counter (Barksdale and Rosenberg 1978).

Results

Figure 1(a-d) shows the stereoselective incorporation of ser into products from the reaction of DL-ser and ImpA in the presence of MepA and imidazole. The four products that contain ser are (a) MepA-ser, (b) ser-ser, (c) ser-N-pA, and (d) unknown-1. Figure 1(a) shows that the formation of MepA-D-ser is preferred over MepA-L-ser. The enantiomeric excess of the D-ser isomer increases slightly from 7% at 1 h to 10% at 12 h, it averages about 9% in the steady state region between 3 h and 12 h. Since MepA-ser is unstable under the conditions of its synthesis, its yield depends on the difference between its rate of synthesis and its rate of decomposition. Its rate of decomposition most likely is the sum of its rates of hydrolysis and aminolysis by ser.

← **Insert Figure 1**

Figure 1(b) shows the formation of ser-ser. In contrast to MepA-ser, there is a preference for L-ser in the synthesis of ser-ser. Early in the reaction at 3 h there is an enantiomeric excess of about 7% of L-ser. This excess decreases throughout the reaction to only 0.4% at 30 h. The synthesis of ser-ser could occur by ser aminolysis of MepA-ser or other intermediates, such as ser-pA and ser-Im, as discussed later.

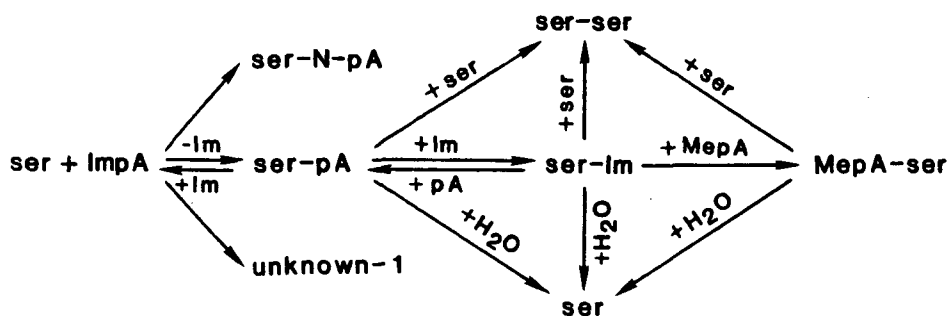
Figures 1(c) and 1(d) show respectively the formation of ser-N-pA and unknown-1. As shown, the formation of D-ser-N-pA is preferred over L-ser-N-pA. The enantiomeric excess of the D-ser isomer averages about 4% throughout the reaction. The synthesis of ser-N-pA, a relatively stable product under the conditions of the reaction, probably involves direct attack of ser on ImpA.

Figure 1(d) shows that unknown-1, which contains ser, also exhibits an enantiomeric excess of D-ser. The excess cannot be accurately estimated because of low yield of this product.

Discussion

The reaction of DL-ser and ImpA in the presence of MepA and imidazole resulted in the stereoselective synthesis of MepA-ser, ser-ser, ser-N-pA, and an unidentified product. MepA-ser formation, which is considered a model of the aminoacylation of tRNA, showed the greatest degree of stereoselection, with about a 9% enantiomeric excess of D-ser. This is believed to be the first case of stereoselection in the synthesis of a 2'(3')-aminoacyl ester of a nucleotide. A similar stereoselection for the D-amino acid had been observed earlier in the formation of the alanyl ester of the internal 2'-hydroxyl group of IpI, and in the related synthesis of the leucyl ester of the 2'-hydroxyl groups of poly(A) (Profy and Usher 1984a, Usher and Needels, 1984). Significant stereoselection (<5%) was not observed in the formation of the alanyl or BOC-alanyl esters of the terminal 2'(3')-hydroxyl groups of IpI (Profy and Usher, 1984a).

The synthesis of MepA-ser most likely occurs via the intermediates, ser-pA and ser-Im, as shown in the reaction scheme below (Weber and Orgel, 1978a). This pathway of MepA-ser synthesis is supported by the identification of glycine adenylate anhydride in similar reactions between glycine and ImpA



(Lohrmann et al. 1975), and the observation that imidazole catalyzes aminoacyl transfer from aminoacyl adenylate anhydrides to the hydroxyl groups of polyribonucleotides (Weber and Lacey, 1975). This type of transfer from an acetyl-aminoacyl adenylate anhydride has been shown to involve an acetylaminoacyl imidazolide intermediate (Lacey and White, 1972). Aminoacyl and acetylaminoacyl imidazolides have been shown to react fairly efficiently with the hydroxyl groups of nucleotides and polynucleotides (Gottikh et al. 1970; Weber and Fox 1973; Profy and Usher 1984a). Decomposition of MepA-ser is shown to occur by hydrolysis and aminolysis by ser (Weber and Orgel, 1979).

The complexity of the reaction network involved in the synthesis of MepA-ser makes it difficult to determine precisely which reactions participate in its stereoselective synthesis. However, an interesting interpretation develops when we consider the stereoselection observed in other products. First, we can eliminate the possible involvement of ser-N-pA and unknown-1 in MepA-ser stereoselection, since their yields are too low to significantly affect the enantiomeric composition of the DL-ser pool which is used in the synthesis of MepA. We can also remove from consideration the hydrolysis of MepA-ser and its aminolysis by DL-ser, because most of the stereoselection of the D-isomer in MepA-ser appears very early in the reaction. The hydrolysis and aminolysis by DL-ser of ser-Im can also be eliminated, since these reactions cannot be stereoselective. This leaves two possibilities. The first possibility is that DL-ser reacts with ImpA to give D-rich ser-pA, which yields D-rich MepA via ser-Im. This does not seem likely, since it would probably give D-rich ser-ser, instead of the L-rich ser-ser we observed early in the reaction when ser-ser synthesis was occurring mostly by serine aminolysis of ser-pA and ser-Im. The increase in the D-isomer content of ser-ser that occurs gradually over the course of the reaction most likely is caused by the increasing contribution of

D-rich MepA-ser in ser-ser synthesis. In fact, most of the synthesis of ser-ser after 9 hours probably occurs by ser-aminolysis of D-rich MepA-ser, since at this time over 85% of the ImpA for ser-pA and ser-Im synthesis has been consumed. The second possibility is that stereoselection occurs in the reaction of ser-Im and MepA to give MepA-ser. If we assume that the reaction of ser-pA with imidazole to give ser-Im is rapid and reversible, then stereoselection of the D-isomer in the synthesis of MepA-ser will result in an excess of the L-isomer of ser-pA and ser-Im. Serine aminolysis of this L-rich ser-pA or ser-Im would then produce L-rich ser-ser, as we observed early in the reaction.

The alternative explanation that formation of L-rich ser-ser brings about the synthesis of D-rich MepA-ser does not seem likely because the initial rate of serine aminolysis to give ser-ser is less than 12% of the rate of MepA-ser synthesis. Although a stereoselective hydrolysis of L-ser-pA to give an excess of D-ser-pA could cause the stereoselective incorporation of D-ser into MepA-ser, it probably does not play a major role, since formation of an excess of D-ser-pA would make the observed enantiomeric excess of L-ser in ser-ser very unlikely. Overall, our results indicate that the selection of D-ser in MepA-ser occurs during the reaction of ser-Im and MepA, that is, during the aminoacylation of a nucleotide.

Prebiotic Significance

In our model of the aminoacylation of tRNA we observed stereoselection of a D-amino acid by a D-nucleotide. This result demonstrates that stereoselection can occur during the non-enzymatic aminoacylation of the 2'(3') hydroxyl groups of a nucleotide. The fact that stereoselection favored the D- rather than the

L-amino acid indicates that the chiral pairing of L-amino acids and D-nucleotides either did not have its origin in the aminoacylation of nucleotides, or that our simple model does not resemble closely enough the early aminoacylation reactions which were responsible for chiral pairing. The latter possibility suggests that modification of the model could result in the stereoselection of L-amino acids by D-nucleotides. Possibly, the use of other 'prebiotic' imidazoles (Oró et al. 1984) or polyribonucleotides in our model would give stereoselection of L-amino acids by D-nucleotides. There are also several other potential sources of chiral selection that could be experimentally studied. They include - peptide bond synthesis (Proby and Usher, 1984a), the binding of amino acid (or polypeptides) to nucleotides (or polynucleotides), and even template-directed synthesis of polynucleotides with nucleoside-5'-phosphorimidazolides (Inoue and Orgel 1983) of chiral peptides containing histidine.

Chiral pairing of L-amino acids and D-ribose of nucleotides could also have originated in other metabolic processes that do not necessarily involve direct interactions between amino acids and nucleotides. For example, the use of non-phosphorylated D-glyceraldehyde and its oxidation product, D-glyceric acid, in early glycolysis would be expected to result in 1) the synthesis of D-ribose instead of L-ribose, and 2) an excess of L-serine, if the early catalyst that carried out serine synthesis by oxidizing D-glycerate to hydroxypyruvate also oxidized D-serine because it could not differentiate D-serine from structurally similar D-glycerate. This type of explanation suggests that the first chiral substance was D-glyceraldehyde (or D-glyceric acid) instead of L-amino acids or D-ribose of nucleotides.

Acknowledgments: This investigation was supported by the National Aeronautics and Space Administration (Grant NSG-7627). I thank Esther Varon for technical assistance in this study.

References

- Barksdale AD, Rosenberg A (1978) Measurement of protein dissociation constants by tritium exchange. In: Hirs CHW, Timasheff SN (eds) Methods in enzymology, vol. XLVIII. Academic Press, New York, p321
- Carter CW, Kraut J (1974) A proposed model for interaction of polypeptides with RNA. *Proc Natl Acad Sci USA* 71:283-287
- Clark VM, Macrae AR, Richter JFP, Todd AR (1966) (N-Phosphoro)- α -amino acids. *Tetrahedron Suppl.* No. 7:307-314
- Gottikh BP, Krayevsky AA, Tarussova NB, Purygin PP, Tsilevich TL (1970) The general synthetic route to amino acid esters of nucleotides and nucleoside-5'-triphosphates and some properties of these compounds. *Tetrahedron* 26:4419-4433
- Greenstein JP, Wintz M (1961) Chemistry of the amino acids, vol.3. John Wiley and Sons, New York, p2202
- Inoue T, Orgel LE (1983) A nonenzymatic RNA polymerase model. *Science* 219: 859-862
- Lacey JC, Pruitt KM (1969) Origin of the genetic code. *Nature* 223:799-804
- Lacey JC, White WE (1972) Aminoacyl transfer: chemical conversion of an aminoacyl adenylate to an imidazolide. *Biochem Biophys Res Commun* 47:565-573

- Lohrmann R, Orgel LE (1978) Preferential formation of (2'-5')-linked internucleotide bonds in non-enzymatic reactions. *Tetrahedron* 34:853-855
- Lohrmann R, Ranganathan R, Sawai H, Orgel LE (1975) Prebiotic peptide-formation in the solid state. *J Mol Evol* 5:57-73
- Mukaiyama T, Hashimoto M (1971) Phosphorylation by oxidation-reduction condensation. Preparation of active phosphorylating reagents. *Bull Chem Soc Jap* 44:2284
- Oró J, Basile B, Cortes S, Shen C, Yamron T (1984) The prebiotic synthesis and catalytic role of imidazoles and other condensing agents. *Origins Life* 14:237-242
- Profy AT, Usher DA (1984a) Stereospecific aminoacylation of a dinucleoside monophosphate by the imidazolides of DL-alanine and N-(*tert*-butoxycarbonyl)-DL-alanine. *J Mol Evol* 20:147-156
- Profy AT, Usher DA (1984b) Stereospecific aminoacylation of polyribonucleotides. *J Am Chem Soc* 106:5030-5031
- Schuber F, Pinck M (1974) On the chemical reactivity of the aminoacyl-tRNA ester bond. I. Influence of pH and nature of the acyl group on the rate of hydrolysis. *Biochimie* 56:383-390
- Usher DA, Needels MC (1984) On the stereospecific aminoacylation of RNA. *Adv Space Res* 4:163-166
- Usher DA, Profy AT, Walstrum SA, Needels MC, Bulack SC, Lo KM (1984) Nucleic acids, proteins, and chirality. *Origins Life* 14:621-628
- Weber AL, Fox SW (1973) Aminoacylation and acetylaminoacylation of homopolyribonucleotides. *Biochim Biophys Acta* 319:174-187
- Weber AL, Lacey JC (1975) Aminoacyl transfer from an adenylate anhydride to polyribonucleotides. *J Mol Evol* 6:309-320

- Weber AL, Orgel LE (1978a) Amino acid activation with adenosine 5'-phosphorimidazole. *J Mol Evol* 11:9-16
- Weber AL, Orgel LE (1978b) The formation of peptides from the 2'(3')-glycyl ester of a nucleotide. *J Mol Evol* 11:189-198
- Weber AL, Orgel LE (1979) The formation of dipeptides from amino acids and the 2'(3')-glycyl ester of an adenylate. *J Mol Evol* 13:185-192
- Zachau HG, Feldmann H (1965) Amino acid esters of RNA, nucleosides, and related compounds. In: Davidson JN, Cohn WE (eds) *Prog Nucleic Acid Res Mol Biol*. Academic Press, New York, p217

Table 1. Chromatographic and electrophoretic mobilities^a

Substance	Relative Mobility			
	System 1	System 2	System 3	System 4
ser	1.00	1.00	1.00	1.00
ser-ser	0.87	0.93	1.45	4.6
MepA-ser	-	0.69	1.28	-
ser-N-pA	0.21	-	0.38	-
MepA	0.98	0.68	0.36	4.31
pA	0.18	0.43	0.36	6.54
Ado	1.44	2.70	1.35	0.22
ImpA	1.32	-	-	-
unknown-1	-	-	0.62	-

^aMobilities are given relative to serine.

Figure 1. Stereoselective reaction of 0.37 M DL-serine and 0.10 M ImpA in the presence of 0.37 M MepA and 2.2 M imidazole at pH 5.8 and 20°C to give (a) MepA-L(and D)-ser, (b) ser-ser(L- and D- residues), (c) D(and L)-ser-N-pA, and (d) unknown-1(L- and D-ser residues). The open symbols are used for measurements up to 6h at which time the reaction and control solutions were frozen at -80°C. After 18h at -80°C, the reaction was continued by bringing the temperature back to 20°C. The solid symbols are used for measurements made after the reaction was restarted.

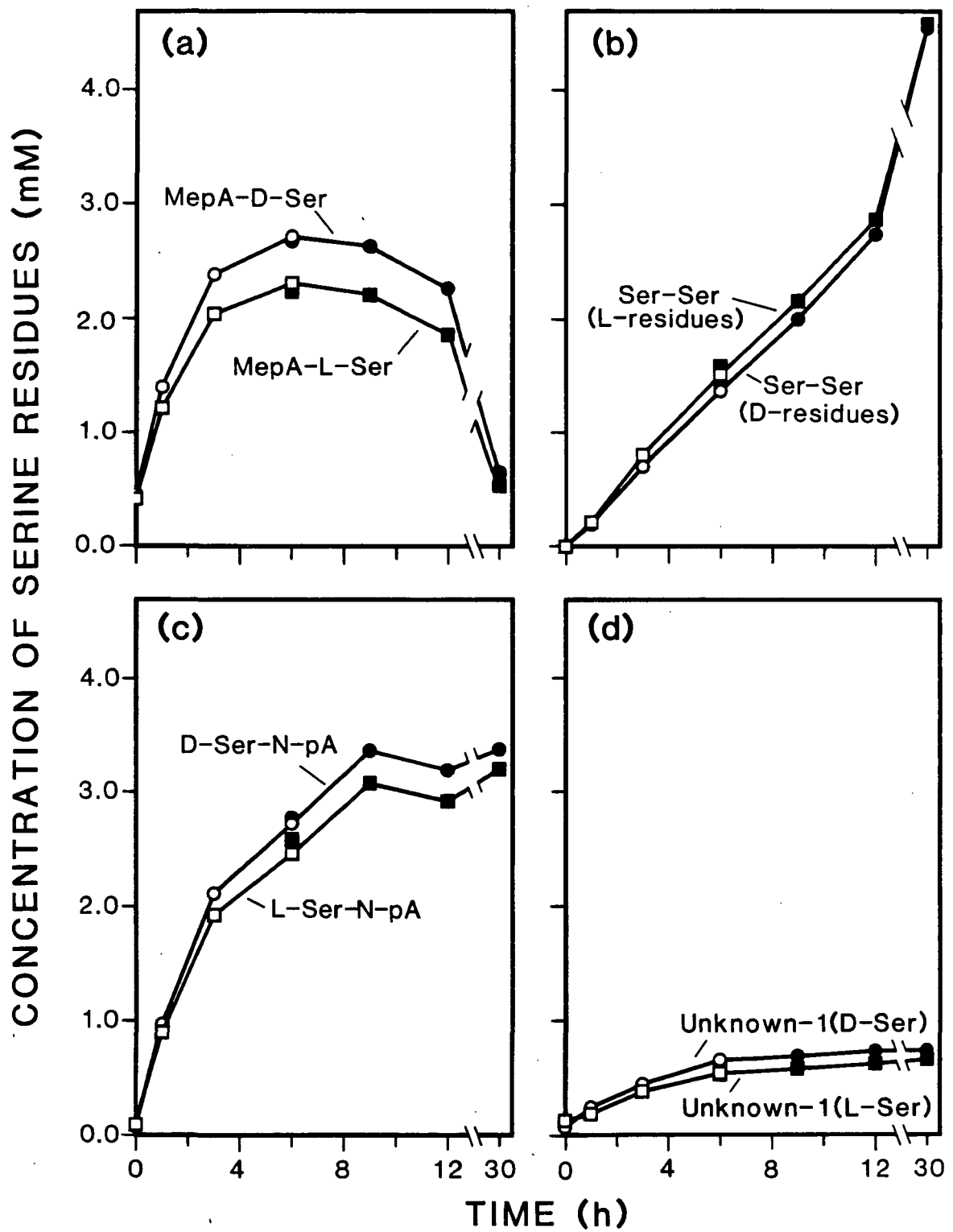


Figure 1

@ NBS

Summary. Reaction of DL-serine and adenosine-5-phosphorimidazolide in the presence of adenosine-5'-(O-methylphosphate) and imidazole resulted in the stereoselective synthesis of the aminoacyl nucleotide ester, 2'(3')-O-seryl-adenosine-5'-(O-methylphosphate). The enantiomeric excess of D-serine incorporated into 2'(3')-O-seryl-adenosine-5'-(O-methylphosphate) was about 9%. Adenylyl-(5'→N)-serine and an unknown product also incorporated an excess of D-serine; however, seryl-serine showed an excess of L-serine. The relationship of these results to the origin of the biological pairing of L-amino acids and nucleotides containing D-ribose is discussed.

@ABA Author.

Key words: Stereoselection - Amino acid activation - Aminoacyl nucleotide ester - Prebiotic chemistry - Origin of optical activity - Nucleotide - Molecular Evolution.

Abbreviations: ImpA, adenosine-5'-phosphorimidazolide; MepA, adenosine-5'-(O-methylphosphate); ser, serine; MepA-ser, 2'(3')-O-(seryl)-adenosine-5'-(O-methylphosphate); ser-ser, serylserine; ser-N-pA, adenylyl-(5'→N)-serine; Ado, adenosine; Im, imidazole; ser-Im, N-(seryl)imidazole; pA, adenosine-5'-monophosphate; ser-pA, seryl adenylate anhydride.